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OBSERVATIONS ON DAILY AND TIDAL RHYTHMS IN SOME FIDDLER CRABS FROM EQUATORIAL BRAZIL [1]

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Experimental studies on daily and tidal rhythmicity in the fiddler crab, *Uca,* have been confined for the most part to North American populations of *U. pugnax* and *U. pugilator*. Both species possess a conspicuous daily rhythm of melanophore activity which consists of diurnal darkening and nocturnal lightening of the integument. This rhythm persists for weeks in the laboratory in darkness, and its frequency is independent of temperature level over a range of at least 20° C. (Brown and Webb, 1948). Superimposed upon the daily rhythm is a persistent tidal rhythm with a mean 12.4-hour period which gives rise to two suppplementary dispersions of melanin during each lunar day of 24.8 hours (Brown *et al.,* 1953). Periodic reinforcement of the maxima of the daily and tidal components when they come into synchrony every 14.8 days produces a semimonthly rhythm of darkening.

The phases of the tidal rhythm are determined by the time at which the crabs emerge from their burrows after being uncovered by the receding tide (Fingerman *et al.,* 1958). Crabs inhabiting burrows near the high tide level emerge earlier than, and show tidal darkening before, crabs living near the low tide level. Accordingly, phase differences have been reported among species (*U. pugilator, U. speciosa* and *U. minax*) inhabiting different levels of the beach (Fingerman, 1956; Fingerman *et al.,* 1958).

In metabolic rate *U. pugnax* and *U. pugilator* each display superimposed diurnal and tidal rhythms which persist in unvarying conditions in the laboratory (Brown *et al.,* 1954). These metabolic rhythms reflect diurnal and tidal rhythms of spontaneous motor activity (Bennett *et al.,* 1957). Generally the maximum of diurnal activity occurs during the early morning hours, and the maximum of tidal activity, near the time of low tide. A review of the studies which describe

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persistent rhythms in *Uca* and which analyze the mechanisms of timing and phase adjustment has been provided by Brown (1962).

Except for the reports of tidal phase differences in melanophore activity, little has been accomplished in the way of a comparative study of persistent rhythmicity at the species level. On the other hand, a framework for such an analysis has been provided by Crane (1941, 1943a, 1943b, 1944, 1957, 1958) in her field studies of behavior in more than fifty species of *Uca* distributed throughout the world. She has reported species differences in the phases of daily and semimonthly rhythms in social behavior. These phase differences are not correlated with habitat as are the species differences in the tidal melanophore rhythm, but rather they appear to be related to phylogenetic position. Furthermore, her descriptions of the diversity of habitats occupied by *Uca* species, ranging from far up tropical rivers to exposed ocean tidal flats, suggest that tidal rhythms must play roles of varying importance in the biology of fiddler crabs. Thus it appears that an extension of the studies on persistent rhythmicity to other members of the genus will yield additional information on the properties of daily and tidal rhythms. At the same time such studies should provide information on the extent to which persistent, or clock-timed, rhythms participate in the regulation of complex behavior patterns.

The following observations were made on some local species of *Uca* in November and December, 1962, during a stay at the Museu Paraense "Emilio Goeldi" in Belém, Brazil. Color change rhythms were observed in *U. mordax, U. rapax, U. maracoani,* and *U. thayeri.* Recordings of locomotor activity were obtained for *U. maracoani* and *U. mordax.*

Collecting Sites

Fiddler crabs were collected in the vicinity of Belém and Salinópolis in the state of Pará in northern Brazil.

Belém is situated on a point of land formed by the entrance of the Rio Guamá into the Rio do Pará, the estuary mouth of the Tocantins, at a distance of about 85 miles from the Atlantic Ocean. The geographical coordinates of the city are latitude 1°27′ S, longitude 48°30′ W. There is a large semidiurnal tide at Belém, the average range of the spring tides being 10.0 feet. Collections of *U. mordax* were made on the southern edge of the city from mud and concrete drainage ditches which empty into the Rio Guamá and which are subject to tidal flooding. Other collections of *U. mordax* were made from the muddy banks of a small tidal stream located a few miles away on the grounds of the Instituto Agronômico do Norte.

Salinópolis is situated on the northern seacoast about 90 miles northeast of Belém. It lies 45 miles from the equator at 0°39′ S latitude, 47°23′ W longitude. To the north of the town is an extensive tidal flat which is separated from the open ocean by offshore shoals. Here the range of spring tides is 15.9 feet. On Nov. 4 *U. maracoani* were collected for activity experiments from the barren mud flat near the middle of the tidal range. On Dec. 9 *U. maracoani* and the following crabs were collected for color change experiments: *U. rapax,* which were picked up near the high tide level where they had burrows in sandy soil among blades of grass; and *U. thayeri,* which were collected from burrows in a rocky clay bank amid tussocks of grass just below the mean high tide level. The habitats resemble ones typically reported for these species.

Rhythms in Color Change

Methods

In the laboratory, crabs were kept in a small amount of water in pans of white enamel or tinted plastic. For exposure to natural changes in illumination, pans were placed outside under the eave of the laboratory building where they were sheltered from direct sunlight and rainfall. Crabs to be maintained in constant light were placed in a windowless room illuminated by incandescent bulbs on a voltage-stabilized power supply. Within the room at the level of the pans light intensity was estimated at a few foot-candles. Air temperature in the room fluctuated around 29° C. with a mean daily range of 3° C.

Stage of melanophore activity was determined with a dissecting microscope according to the Hogben and Slome (1931) scale for describing the degree of dispersion. On this scale stage 5 indicates complete dispersion and stage 1, complete concentration. Melanophores on the anterior aspect of the merus of the first or second walking leg were observed. In *U. thayeri,* unlike the other species, the melanophores generally remained discrete and were seldom seen to coalesce. In addition, the stages of individual melanophores usually varied widely over the surface of the same leg. For this species only the central area of the merus of the second leg was staged. In the figures to follow each value represents the arithmetic mean of the sample. The size of samples varied because of availability of crabs. Both males and females were used.

U. mordax were collected from the Instituto Agronômico on Nov. 20 and divided into two lots, one being exposed to natural changes in illumination and the other to constant illumination. Melanophores were staged through the day in both lots on Nov. 26.

On Dec. 9 crabs collected at Salinópolis were sorted into pans and placed outdoors in natural light with the *U. mordax* remaining from the collection of Nov. 20. Starting at 4 AM on Dec. 12 all groups were staged at two-hour intervals until 8 PM on Dec. 13. On the morning of Dec. 14 the pans were placed in the constant-light room, and at 6 PM on Dec. 15 staging was begun and continued at six-hour intervals until 12 PM on Dec. 19. By Dec. 15 the original collection of *U. mordax* had so dwindled that it was replaced by a new lot of *U. mordax* collected at 5 PM on that day from the drainage ditches along the Rio Guamá. Standard time for Belém is used; the time meridian is 45° W.

Results

In Figure 1 are compared the average hourly stages of melanophores in two lots of *U. mordax* collected six days earlier, on Nov. 20, at the Instituto Agronômico. In the lot which had been exposed throughout to natural illumination, the rhythm of pigment migration passes through nearly the full range of concentration and dispersion. Periods of transition are sharp and occur at the times of sunrise and sunset. In the second group of crabs, which had been kept in constant low illumination, the rhythm of color change has been modified. The range of dispersion from mean minimum to mean maximum is much reduced; the periods of transition are very gradual; and the maximum dispersion no longer occurs during the daylight hours.

In Figure 2 are presented the daily rhythms in natural illlumination for three species from Salinópolis and the *U. mordax* still remaining from the collection of Nov. 20. Both *U. rapax* and *U. maracoani* possess overt rhythms whose general features are similar to those of *U. mordax*. By contrast the rhythm in *U. thayeri* is of very low amplitude.

During the staging it was obvious that the red chromatophores of *U. rapax* and *U. maracoani* were going through a daily variation roughly parallel to that of the melanophores. In *U. rapax* the red chromatophores were very evident, being numerous and resembling the melanophores in their dimensions.

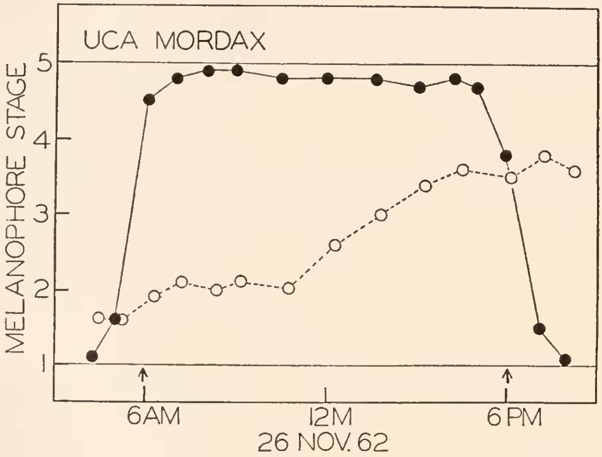

Figure 1. Melanophore rhythms in *U. mordax* after six days in experimental conditions. Closed circles are the average of 44 animals in natural illumination. Open circles are the average of 50 animals in constant low illumination. Arrows indicate times of sunrise and sunset at Belém.

The behavior of melanophores following transfer to constant low illumination is shown in Figure 3. The rhythm of color change persists for at least four days in *U. mordax* and six days in *U. rapax* and *U. maracoani*. In *U. thayeri* the amplitude is so much reduced that a rhythm is scarcely discernible.

The overt rhythms of *U. mordax, U. rapax,* and *U. maracoani* are common in several ways. In all cases the range of daily pigment migration is reduced below its range in natural illumination. For all three species the maximum and minimum levels of dispersion reached on each day remain fairly constant throughout the period of observation. Finally, inspection of the figure discloses that rhythms for two species have drifted out of phase with the outside daylight-darkness cycle. For *U. rapax* the maximum has shifted from noon to 6 PM in about six days. In *U. mordax,* where a similar phase shift has occurred, the results are essentially a repetition of those obtained in the earlier experiment (see Fig. 1), except for a change in amplitude.

Differences among the species occur in the daily levels of pigment dispersion and concentration. In *U. mordax* the melanophores fail to concentrate fully during the first night in constant light and for the following nights, but more apparent is their failure to disperse during the day phase. For *U. rapax* the melanophores fail to concentrate fully but do achieve nearly the maximum of dispersion observed in natural illumination. In *U. maracoani* there is failure both to concentrate and to disperse fully.

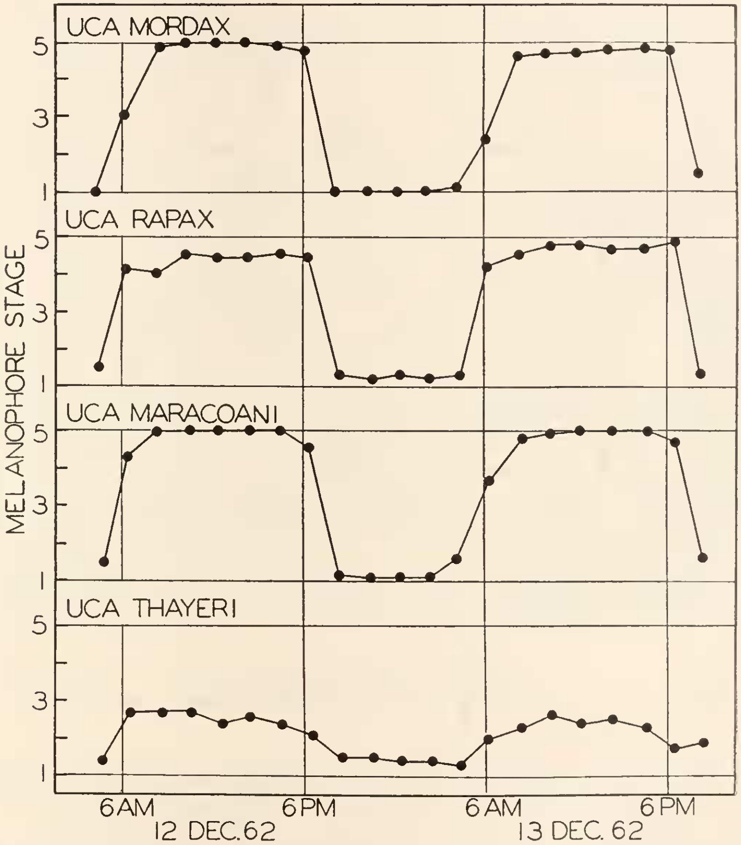

FIGURE 2. Melanophore rhythms in natural illumination. Number of crabs used were the following: *U. mordax*, 22; *U. rapax*, 30; *U. maracoani*, 26; and *U. thayeri*, 29.

Discussion

The overt rhythms of pigment migration in *U. mordax, U. rapax,* and *U. maracoani* exposed to natural changes of illumination are closely adjusted to the equatorial cycle of daylight and darkness, suggesting that the rhythms serve a camouflaging and protective function. *U. thayeri* does not exhibit an overt rhythm of color change, but it is notable that this species is characterized by patches of pile on the dorsal surface of the carapace and appendages. Possibly this morphological covering substitutes functionally for the melanophore rhythm.

The red chromatophores of *U. rapax* and *U. maracoani* undergo a daily rhythm of expansion and contraction when the crabs are exposed to natural cycles of illumination or to continuous low lighting. A daily rhythm has also been reported for the red chromatophores of *U. pugilator* (Brown, 1950). However, the rhythm in *U. pugilator* is evident only in uninterrupted darkness, since under conditions of illumination the stage of the red chromatophores depends primarily upon background. It is interesting that this rhythm is not salient in *U. pugnax* because until recently *U. rapax* and *U. pugnax* had been regarded as subspecies (Tashian and Vernberg, 1958). The rhythm in red chromatophore activity is thus a physiological distinction between the two groups.

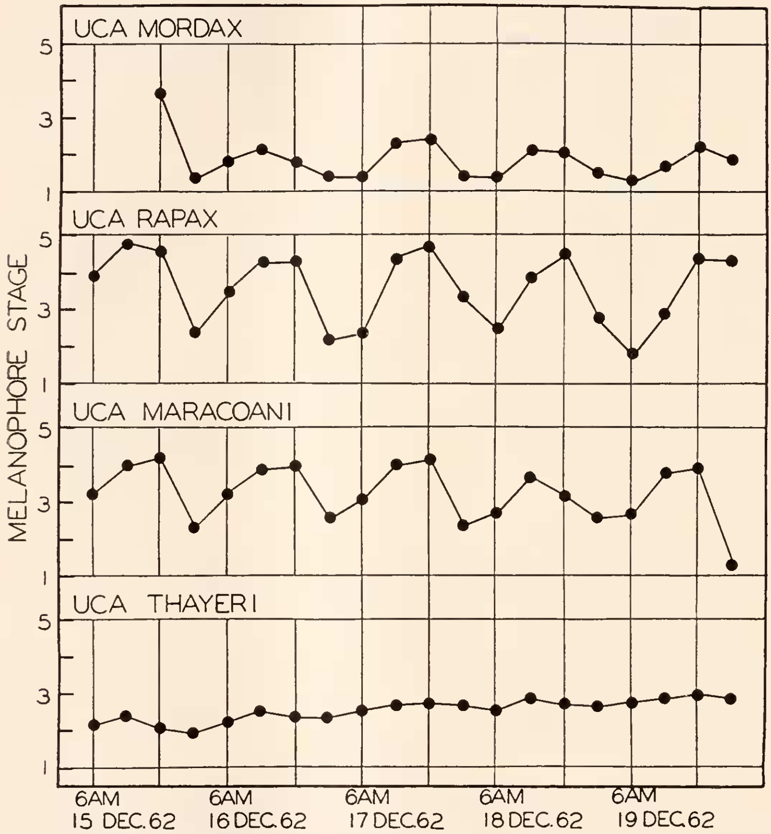

FIGURE 3. Melanophore rhythms in constant low illumination. Number of crabs used decreased during the experiment from 31 to 9 in *U. mordax,* 27 to 12 in *U. rapax,* 25 to 7 in *U. maracoani,* and 25 to 12 in *U. thayeri.*

Constant illumination clearly modifies the expression of the melanophore rhythm. Although the data of this study are very limited it is possible to make some comparisons with the results of a detailed series of experiments on the behavior of

U. pugnax melanophores in constant illumination (Brown and Hines, 1952). In *U. pugnax* constant illumination reduces the amplitude of the rhythm by inhibiting concentration during the night phase. For crabs on a white background the inhibition occurs progressively over several days. On a black background the maximum inhibition is achieved on the first day of the experiment.

The rhythm of *U. mordax* in constant light fails to resemble any pattern obtained for *U. pugnax* by Brown and Hines (1952). On the other hand, the suppression of the phase of diurnal dispersion in *U. mordax* is similar to the response of *U. pugnax* when it is transferred from a daily schedule of alternating light and darkness to uninterrupted darkness (Brown and Stephens, 1951). In *U. pugnax,* however, the initially inhibited diurnal dispersion regained its amplitude progressively over a period of days. This same phenomenon was observed also in a preliminary experiment with *U. mordax,* in which recovery of the amplitude of the rhythm was recorded over three consecutive days after the crabs had been placed in darkness. In *U. maracoani* the form of the rhythm resembles the one eventually attained by *U. pugnax* after several days at low light intensity on a white background. Whereas both *U. mordax* and *U. maracoani* were kept in white pans, *U. rapax* were kept in a green-tinted container. The response of these latter crabs resembles that obtained with *U. pugnax* on a black background at a light intensity of two-foot candles. In summary, constant illumination reduces the amplitude of the melanophore rhythm in fiddler crabs so far examined; but species differences occur in the manner by which reduction is effected.

In constant illumination the period of the melanophore rhythm in *U. mordax* and *U. rapax* exceeds 24 hours. A similar increase in period has been reported for *U. pugnax* in constant low illumination (Webb *et al.,* 1954). Deviation from a 24-hour period is commonly observed in many physiological rhythms when they are studied under constant illumination and temperature. The occurrence of these unnatural periods has been interpreted as an artifact of laboratory conditions produced by operation of the adaptive mechanism which ordinarily serves to adjust phases of the rhythm to natural light or temperature cycles (Brown, 1962).

Rhythms in Motor Activity

Methods

Spontaneous motor activity was recorded with an actograph consisting of tipping pans and a mechanical recording system somewhat similar to the one described by Bennett *et al.* (1957). On the evening of the day of collection crabs were placed individually with a few milliliters of water from the collecting site in aluminum pans with transparent plastic covers. Dimensions of the pans were 12 cm. in length, 6 cm. in width, and 4 cm. in height. The actograph was set within a small inner room of the laboratory where the recorded temperature averaged 30° C. and did not vary through more than 1° C. during any 24-hour period. The intensity of illumination was less than one foot-candle and was constant since the power was supplied from a voltage-regulated source. Activity of ten individuals was recorded simultaneously on a kymograph drum moving at the rate of 10 mm. per hour. Activity was measured from the records as millimeters of oscillation of the tipping pan per hour, and for each hour the average activity of all animals

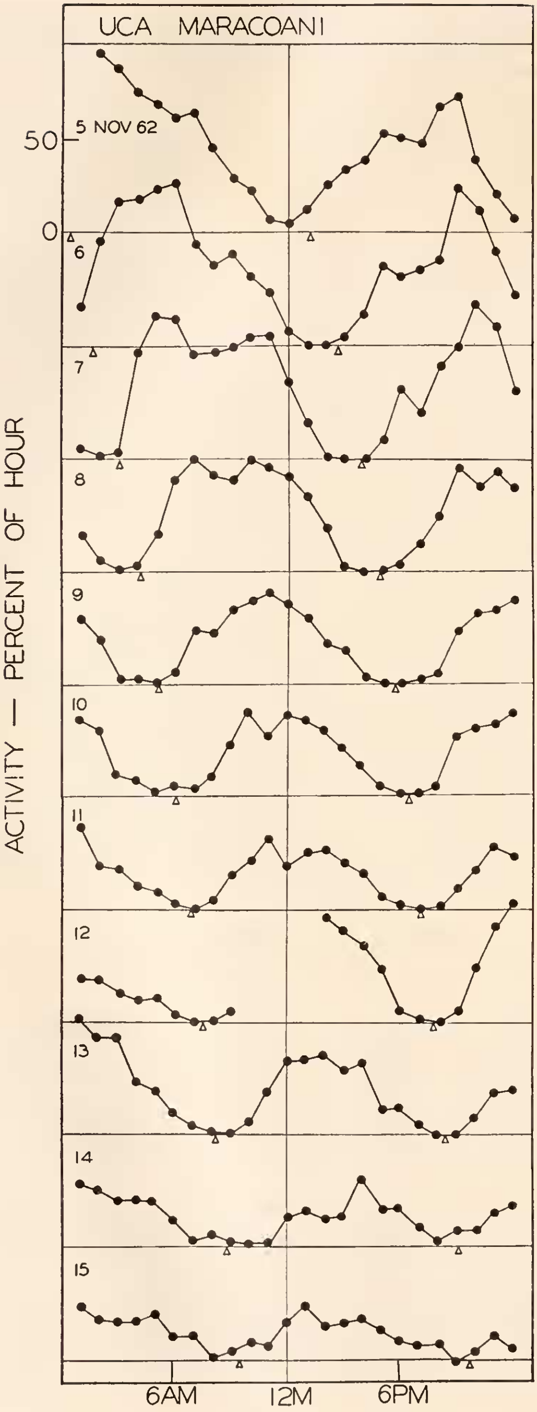
UCA MARACOANI
ACTIVITY — PERCENT OF HOUR
50
0
5 NOV 62
6
7
8
9
10
11
12
13
14
15
6AM
12M
6PM

FIGURE 4.

was computed. The activity was expressed as a percentage referring to the fraction of time in each hour that crabs were active. Each hourly value represents the amount of activity recorded during the preceding hourly interval; for example, the value for 6 AM is the measure of activity between 5 and 6 AM.

U. maracoani were collected at Salinópolis on Nov. 4 and recorded from Nov. 5 through Nov. 15. *U. mordax* were collected from drainage ditches along the Rio Guamá on Nov. 23 and recorded between Nov. 24 and Dec. 7. In both cases only large adult males were used. On Nov. 12, at the time of a power failure, the sea water in the pans of *U. maracoani* was changed. Except for this instance the crabs remained sealed in their containers throughout the period of recording.

Results

In Figure 4 are presented the mean hourly values recorded for *U. maracoani*. In its spontaneous activity *U. maracoani* possesses an overt tidal rhythm. On each day during the 11 days of study there occur two maxima and two minima which are synchronized with tidal events at Salinópolis. In the figure are indicated the times of high tide to which the minima of the activity cycle are closely tied. Although the amplitude of the activity cycles gradually decreases during the recording period it should be noted that this is due primarily to reduction in the height of the tidal maxima. The periods of almost complete inactivity at the time of high tide do not tend to disappear but are still present during the final days of recording. If the phases of individual animals had tended to drift apart the well defined periods of inactivity would have become obscured. Thus, there is no apparent loss of phase synchrony during 11 days among the ten animals under study. On Nov. 12 when the sea water in the aluminum pans was changed, there was a recovery for two or three cycles from the trend of decreasing amplitude.

It is often stated that the rate of tidal progression is 50 minutes per day, meaning that this is the interval between the recurrence of tidal events on successive days. Generally it is realized that this represents a mean value from which the actual interval may depart widely. As an example, on the first three days of the activity study, Nov. 5, 6, and 7, the average rate of progression of high tides at Salinópolis was 80 minutes per day. For the last five days, Nov. 11 through 15, the average rate had decreased to 41 minutes per day. Study of Figure 4 discloses that the crab activity cycles were also progressing across the day more rapidly at the beginning of the study than at the end, suggesting that the persistent activity of *U. maracoani* was following the actual rate of tidal progression rather than an average rate of 50 minutes per day.

In Figure 5A are presented the mean daily activity patterns for *U. mordax*. The recorded activity of these crabs is more variable than in the preceding case, and so, to facilitate analysis, the mean curves for each day have been smoothed with three-hour overlapping, or moving, averages. From inspection of the figure it is evident that the daily patterns for *U. mordax* are more complex than those of

FIGURE 4. Average daily patterns of spontaneous motor activity in *U. maracoani*, from November 5 through November 15, 1962. Values are expressed as the percentage of the hour that crabs were active. Triangles indicate times of high tide at Salinópolis where the crabs were collected. All tidal times were obtained from Tide Tables (1962) of the Coast and Geodetic Survey.

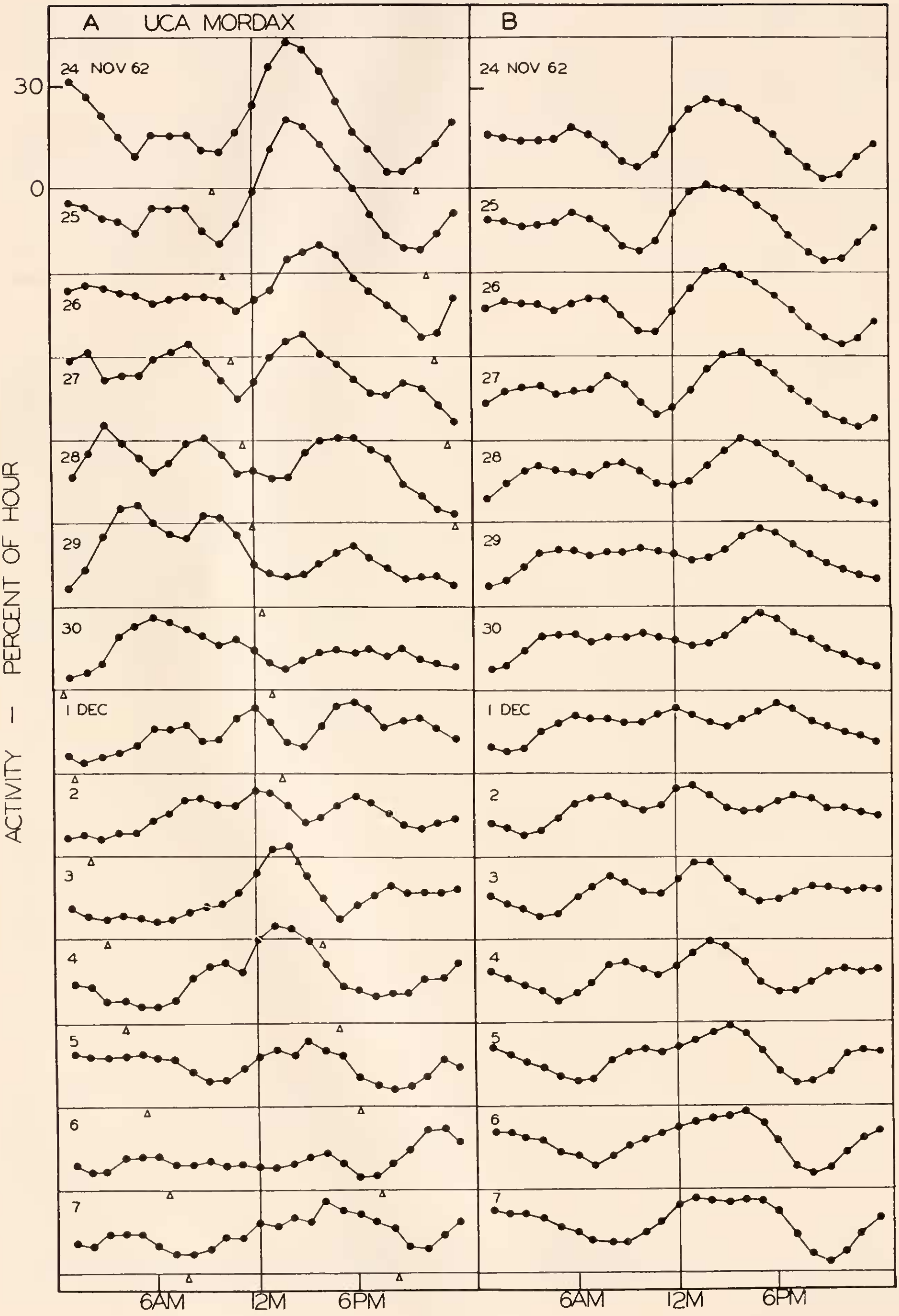
A
UCA MORDAX
B
24 NOV 62
25
26
27
28
29
30
1 DEC
2
3
4
5
6
7
30
0
ACTIVITY — PERCENT OF HOUR
6AM
12M
6PM

FIGURE 5.

U. maracoani. On the first two days of recording a large afternoon maximum occurs at the time of low tide, and two minima occur near the times of high tide. Thereafter the progression of a shifting, or phase-labile, component may be traced through successive daily patterns. For instance, one can follow the minimum which occurs at 9 to 10 AM on Nov. 24 and 25. On Nov. 27 the minimum is centered at 11 AM; on Nov. 29, at 2 PM; on Dec. 1, at 3 PM; on Dec. 3, at 5 PM; on Dec. 5, at 8 PM; and on Dec. 7, at 10 PM.

During the first five days of recording the average rate of progression for high tides at Belém was 36 minutes per day. Between Dec. 3 and 7 the average rate had increased to 67 minutes per day. It is clear from examination of Figure 5A that the phase-labile component of the activity rhythm is progressing slowly during the first days of the study and more rapidly during the period when the rate of actual tidal progression is increased. This suggests that the phase-labile component is a persistent tidal rhythm which is following the true rate of tidal progression.

Since approximately a semimonthly period of data is available, an analysis can be made to separate any components of diurnal and lunar frequency in order that their form and phase relationships may be examined. The method is essentially one which has been used for the determination of lunar variations in geophysical data. Its application to the analysis of rhythms in semimonthly periods of metabolic activity in *Uca* has recently been discussed (Webb and Brown, 1961). Briefly, the mean *diurnal,* or *solar daily,* rhythm is obtained by averaging the amount of activity for each hour of the day for the whole 14-day period. Because all phases of the tidal component will have occurred during each hour, their effects upon the mean diurnal rhythm will have been largely randomized. To obtain the mean *lunar daily* rhythm the mean diurnal curve is first subtracted from each of the original 14 days of data to leave the residual lunar component. The hours of the day so treated are next rearranged according to lunar time and averaged. Lunar rearrangement was accomplished by synchronizing for all days the hour on each during which the moon passed through the upper meridian at Belém. The time of upper transit was computed from the American Ephemeris and Nautical Almanac (1962).

As already demonstrated, the actual rate of progression of tidal events varies widely about its mean rate. This variation also occurs with respect to the relatively more uniform rate of progression of the time of upper transit. During the period of study with *U. mordax* the times of high tide ranged from 33 to 120 minutes before the moment of upper transit. Therefore, in synchronizing the times of upper transit one does not perfectly synchronize the times of occurrence of tidal events. This is indicated in Figure 6 where the mean diurnal and lunar components of activity are presented.

The lunar component (Fig. 6B) in motor activity of *U. mordax* resembles the overt tidal rhythm in *U. maracoani* in that maximum activity occurs during the times of low tide, and minimum activity at the times of high tide. *U. mordax,*

FIGURE 5. A. Average daily patterns of spontaneous motor activity in *U. mordax,* from November 24 through December 7, 1962. Triangles indicate times of high tide at Belém where the crabs were collected. B. Daily patterns obtained by combining the mean solar and lunar components of activity in *U. mordax.*

however, differs from *U. maracoani* in possessing a large diurnal component (Fig. 6A) comprised of a broad afternoon maximum and an evening minimum. The range from lowest to highest values of the mean diurnal curve, expressed as percentage of the hour that the crabs are active, is from 10.1 to 21.1, or 11.0. This is slightly exceeded by the range of the lunar variation, which is 12.8.

Because the preceding analysis distinguishes only components of approximately lunar and solar frequency, it is of interest to determine to what extent these components alone can account for day-to-day variations in the patterns of activity in *U. mordax*. For this purpose Figure 5B has been prepared. This figure presents a recombination of the mean solar and lunar variations (see Fig. 6) in their natural phase relationship for each day of the activity study. On Nov. 24, for example, upper transit occurred between 10 and 11 AM. On this day the value for the hour of upper transit in the lunar component was synchronized with the 11 AM value

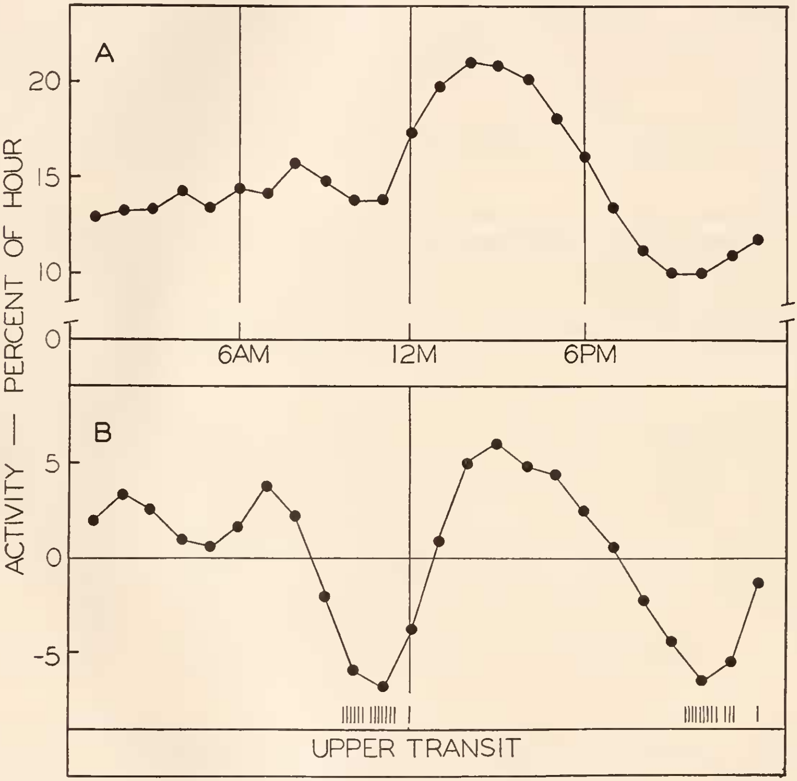

FIGURE 6. A. Mean diurnal component of spontaneous activity in *U. mordax* for the period from November 24 through December 7, 1962. B. Mean lunar component of motor activity in *U. mordax* during the same period. Short vertical lines represent hours of occurrence of incompletely synchronized high tides. Six high tides occurred two hours before upper transit; seven occurred one hour before; and one occurred during the hour of upper transit. Distribution of tides near the time of lower transit is indicated in a similar manner.

for the mean solar component and the two curves were added algebraically. It is thus possible in Figure 5 to compare for each day the pattern of actual recorded activity with the pattern computed from the mean solar and lunar components. More obvious differences between the two sets of curves appear on Nov. 28 through 30 when the relative amplitudes of the morning and afternoon maxima are reversed between actual and computed patterns. Nevertheless, it is clear that comparable major maxima and minima are present at the same times of day for both sets of curves. On Dec. 3 the morning peak in the recorded activity is not conspicuous although its presence is suggested. On Dec. 5 the morning peak of recorded activity appears inverted. On Dec. 6 the maximum occurring over the noon hour is not expressed although the 4 PM peak is present. Aside from these points there is good agreement between the recorded and computed activity. The hour-by-hour correlation between the two sets of data for the entire 14-day period has a coefficient, r, of $+0.64$. The coefficient of determination, or r^2, is 0.41. Thus 41% of all variation in the activity of *U. mordax* presented in Figure 5A is accounted for in terms of mean diurnal and lunar components. This simple comparison does not take into account the progressive decline in mean level of activity which occurs during the recording period. This decrease involved a reduction of the mean daily activity by almost 50% between the beginning and end of the study. As a consequence the patterns of activity computed from the mean solar and lunar components underestimate the actual level of activity during the first days of recording and overestimate it during the final days. Considerable variation is thereby introduced into the correlation between the two time series and results in a substantial reduction of the coefficient of correlation.

From the foregoing, then, it is evident that the mean lunar and solar components account quite well for major variations in the form of the actual recorded data from which they were statistically derived. Clearly the two components cannot be dismissed as statistical artifacts.

Discussion

Continuous recordings of motor activity or metabolic rate have now been obtained with four species of *Uca*. The persistent rhythm in *U. maracoani* is singular in possessing only an overt tidal component. On the other hand, the rhythm of *U. mordax* resembles rhythms already described for *U. pugnax* and *U. pugilator*. Similarities among the three species are the following. The activity can be resolved into lunar and solar components; the amplitude of the lunar component is equal to, or exceeds, that of the solar; and the major maxima of the lunar component occur near the times of low tide. However, in *U. mordax* the form of the diurnal component differs from that which has been obtained for *U. pugnax* and *U. pugilator* (Brown, 1960), with one exception. In the summer of 1954 the diurnal components in the metabolic rhythms of both *U. pugnax* and *U. pugilator* were essentially inversions of the usual pattern, and they resemble closely the form of the diurnal component of *U. mordax*.

This latter case directs attention to difficulties in attaching ecological significance to the form of rhythmic patterns recorded under laboratory conditions. The atypical form of the 1954 metabolic cycles was subsequently found to be correlated with concurrent inversions of certain geophysical cycles (Brown *et al.*, 1958). Although a number of correlations have been described between geophysical variations and varations in metabolic rate in *Uca* maintained under controlled conditions, it is not known to what extent these have any significance in determining the form or phase of adaptive rhythmic behavior in natural conditions. It has been clearly recognized (Bennett *et al.,* 1957) that activity patterns recorded in the laboratory are influenced by a number of factors including pervasive geophysical forces and, of course, the artificial conditions of the experimental situation.

In connection with the preceding discussion it may be recalled that practically all fiddler crabs have been thought to be diurnal (Crane, 1958), and yet the persistent rhythm of *U. maracoani* collected from a typical habitat shows no conspicuous diurnal component. Even in species with a diurnal component, the persistent tidal cycles of activity are still present during night-time hours. If these crabs are in fact diurnal, this would indicate that the persistent rhythms recorded in the laboratory must be extensively modified in the field by other factors, including possibly the daily cycle of light and darkness. On the other hand, the occurrence and possible extent of nocturnal activity in *Uca* species have recently been discussed (von Hagen, 1962). Such activity has been documented for *U. pugilator* (Burkenroad, 1947) and *U. tangeri* (von Hagen, 1961). A further example is the finding that *U. pugnax* and *U. pugilator* in the Woods Hole area of Massachusetts were out of their burrows and actively feeding when low tide occurred at 2 AM on a moonless night in July (Webb and Barnwell, 1963, unpublished observations).

With *U. maracoani* a closer comparison can be made between the laboratory results and field observations. The behavior patterns of this species have been studied in the field and under near-natural conditions in a "crabbery" by Crane (1958). In the crabbery it was necessary to simulate tidal flooding in order to maintain the normal level of activity and social behavior, including waving and display of the males; but when water level was held constant signs of a tidal rhythm persisted for at least several days. In the light of the laboratory study, this might be expected. However, also present in the field and under tidal conditions in the crabbery were diurnal and semimonthly cycles of social behavior whose timing was largely independent of day-to-day fluctuations in the level of daytime illumination and temperature. These diurnal and semimonthly rhythms were not evident in the present study, in which the tipping pan activity of isolated *U. maracoani* was recorded. Probably the daily rhythm of social behavior was not expressed at all under such conditions, or, even if it were present, it might not be reflected in a recording of spontaneous motor activity.

With the aforementioned reservations in mind it may be suggested that general features of the persistent rhythms in *U. maracoani* and *U. mordax* are related to their respective habitats. *U. maracoani* were collected from burrows on a barren expanse of mud flat near the middle of the tidal range. In this species the persistent activity pattern, an overt tidal rhythm, is correlated with an environment dominated by tidal ebb and flow. *U. mordax* were picked up from the middle and upper levels of drainage ditches along the Rio Guamá, a typical habitat for the species which is the common fiddler crab of tropical rivers and streams of the western Atlantic. This crab has been reported in burrows so far back from the river's edge that it is only reached by the semimonthly spring tides (Crane, 1943). In a species whose activity is less dependent upon the tides, it is not surprising to find a large diurnal component in addition to the tidal component.

The results of experiments with *U. maracoani* and *U. mordax* suggest that the persistent tidal component of their activity rhythms follows the actual variations in the rate of progression of tidal events. This suggestion is based upon correlations between spontaneous changes in frequency of activity cycles and changes in the rate of tidal progression. These correlations occurred during a period when the rate of tidal progression decreased in *U. maracoani* and increased in *U. mordax*. The earlier studies on tidal melanophore rhythms (Fingerman *et al.*, 1958) demonstrated a close adjustment in the phasing of the biological cycle to the tidal cycle. The present activity experiments suggest that there may also be a biological adjustment to the actual systematic variations in tidal frequency.

The hypothesis presented here to account for frequency changes in the tidal rhythms of activity is that the rhythms are timed by the interaction of solar and lunar components. When rhythms of solar and lunar frequency are combined, the solar component tends to displace the times of occurrence of maxima and minima of the lunar component. Consequently, as it progresses across the peaks and troughs of the solar curve, the lunar component appears to deviate around the lunar rate of progression by systematically increasing and decreasing its frequency. In *U. mordax,* variations in frequency of the apparent lunar component are an expected result of the simultaneous occurrence of the solar and lunar components which have been demonstrated for this species. This effect is evident in Figure 5B where the minimum, occurring at about 10 AM on Nov. 24, shifted only two and one-half hours during the first five days but shifted six hours during the next six days from Nov. 29 through Dec. 4. In *U. maracoani* a diurnal component is not expressed. Instead, during each lunar day there are two peaks of approximately equal amplitude whose timing appears to be adjusted to the timing of tidal events at the beach of collection. According to the hypothesis, the pattern of overt behavior would be timed by an underlying solar and lunar interaction. That *U. maracoani* actually does possess the means for measuring accurately a daily period was shown by the persistent daily melanophore rhythm.

This hypothesis does not depend upon whether the timing information which runs the biological clock system is of endogenous or exogenous origin. Possibly the two rhythms of lunar and solar frequency could be produced by an autonomous, internal biological timer. On the other hand, it is equally possible that the organism derives information about the lengths of these natural periods through response to rhythmic geophysical factors in its environment. In the latter case geophysical rhythms would provide information only about frequency and not about phase. Phase would be determined adaptively by factors in the local tidal environment.

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Summary and Conclusions

1. Experimental observations were made on daily and tidal rhythms in four species of fiddler crabs, *Uca,* collected at Belém and Salinópolis near the equator in Brazil.

2. In naturally changing illumination, overt daily rhythms of melanophore activity were present in *U. mordax, U. rapax,* and *U. maracoani.* The form of the rhythms was related to the equatorial cycle of daylight and darkness.

3. There was a melanophore rhythm in *U. thayeri,* but its amplitude was less than one-third of the amplitude of the other species. In *U. thayeri,* as in *U. pugilator,* the melanophores failed to coalesce during the diurnal phase of dispersion.

4. The red chromatophores of *U. rapax* and *U. maracoani* displayed daily rhythms of concentration and dispersion.

5. In constant illumination the amplitude of the melanophore rhythms was reduced. In at least two of the species, *U. mordax* and *U. rapax,* the melanophore rhythm deviated in period from 24 hours.

6. In its spontaneous motor activity *U. maracoani* possessed a tidal rhythm which persisted in constant illumination and temperature. *U. mordax* had a more complex and variable pattern which could be resolved into persistent solar and lunar components.

7. Differences in the activity patterns of the two species appeared to be related adaptively to differences in their habitats.

8. The frequency of the persistent tidal rhythms seemed to follow the actual systematic variations in frequency of the tidal cycle. Such deviations about a true lunar frequency would be expected if the tidal rhythms were timed by two components, one of solar and the other of lunar frequency.

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